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Studies on Synergist for Insecticides. (XXVII) On the Synergistic Effect of Several Lignans on Pyrethrins and Allethrin

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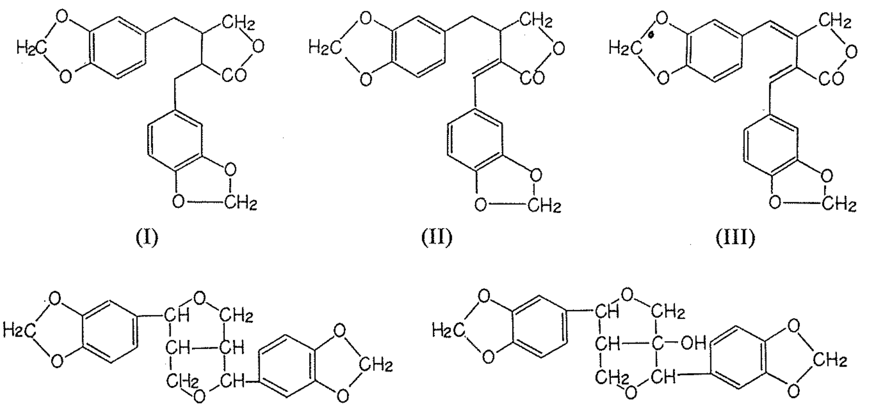
The comparative value of several lignans such as hinokinin, hibalactone, taiwanin A, *d*-sesamin and pawlownin as synergists with either pyrethrins or allethrin was estimated in tests of the lethal and knockdown effectiveness against houseflies, *Musca domestica* L. by the topical application or settling dust apparatus method, and against larvae of the common house mosquito, *Culex pipiens pallens* Coqui. by the petri-dish method. Taiwanin A was less synergistic with not only pyrethrins but allethrin than hinokinin or hibalactone, and also pawlownin was less synergistic with pyrethrins and allethrin than *d*-sesamin. The substitution of two double bonds or one hydroxyl group into the carbon chain or bis-furan central nucleus between two of 3,4-methylenedioxyphenyl groups causes the decrease in synergistic activity.

INTRODUCTION

On the synergistic effect of several lignans containing 3,4-methylenedioxyphenyl group such as *l*-asarinin, hinokinin (I), egonol and hibalactone (savinin) (II) on pyrethrins and allethrin has been reported in the previous papers.[1] Recently, new two compounds taiwanin A (III) and pawlownin (V) which were closely connected with above mentioned lignans structurally, were found.



(I) (II) (III) (IV) (V)

Chart 1. The chemical structure of lignans tested.

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The former was isolated from heartwood of *Taiwania cryptomerioides* Hayata (Japanese name "Taiwansugi") by Lin *et al.*[2] and the latter was also isolated from wood of *Pawlownia tomentosa* Steud. (Japanese name "Kiri") by Takahashi *et al.*[3]

The comparative synergistic activities of hinokinin, hibalactone, taiwanin A, *d*-sesanin (IV), pawlownin and piperonyl butoxide with pyrethrins and allethrin, and furthermore, the relation between chemical structure and their synergistic activities were studied in the present work.

Formerly, Yamashita *et al.*[4] were investigated on the synergistic action of synthetic phenolic lactone such as (±) hinokinin, (±) hibalactone, α-piperonyliden-γ-butyrolactone and α-benzyliden-γ-butyrolactone with pyrethrins and allethrin. But they did not study for lignans occurring naturally and used only rice-weevil as a test insect. At the present work, houseflies and mosquito larvae were used as test insects and evaluated for the lethal and knockdown effectiveness against both insects.

EXPERIMENTAL

Materials and Methods

Test sample

The standard pyrethroids employed in the experiment were 20% pyrethrum extract (pyrethrin I 10.8%; pyrethrin II 9.2%) prepared by Dainippon Jotyugiku Co. Ltd., and pynamin containing 92% of allethrin prepared by Sumitomo Chemical Co. Ltd.. Hinokinin, mp 63–64°, was isolated from the heartwood of *Chamaecyparis obtusa* Sieb. et Zucc. (Japanese name "Hinoki") by means of Keimatsu *et al.*'s method[5] and hibalactone, mp 145.0–146.0°, was also isolated from new leaves of *Chamaecyparis obtusa* Sieb. et Zucc. (Japanese name "Chabohiba"; variety of "Hinoki") by means of Masumura's method.[6] Taiwanin A was supplied by Lin, and the mixture of crude pawlownin and *d*-sesamin extracted by methanol from wood of *Pawlownia tomentosa* Steud. was separated to pure two crystals (mp 104–105°; 121.5–122.5°) individually by silica gel chromatography. The sample of technical piperonyl butoxide used contained 85.6% of 3,4-methylenedioxy-6-propylbenzyl butyl diethyleneglycol ether. Triton X-100 (polyoxyethylene t.-octyl phenol ether) was used as emulsifier of stock emulsions of pyrethroids alone or with synergists.

Test insects

Adult houseflies, *Musca domestica* L. (Lab. em. 7, em. strain) susceptible to insecticides were kept at 25° and 70% relative humidity and were fed on sucrose, powdered milk and water, only female, 4–5 days old, were used in the experiment. Larvae of the common house mosquito, *Culex pipiens pallens* Coqui. susceptible to organophosphorous and organochlorinated insecticides were fed on dry yeast in the glass jar and only last inster were used.

Test methods

Topical application against houseflies. The synergists, dissolved in acetone, was combined with pyrethroids at a ratio of 8:1. Each solution was tested at four or five concentrations, approximately 100 female flies were used for each concentration. Houseflies were each dosed by measured drop technique. The kill was usually recorded 24 hrs. after treatment.

Knockdown test against houseflies. Acetone or ether solution of pyrethroids combined with synergists at a ratio of 1:8 was added to talk dust in a flask and solvent was evaporated off, residual dust was pulverized to 200 mesh powder in a mortar. The knockdown effectiveness of these synergized pyrethroids dust against houseflies was evaluated by means of settling dust apparatus method of Nagasawa.[7] About 100–130 individuals were used in each test.

Lethal test against mosquito larvae. The composition of the stock emulsion used for lethal effectiveness in the experiment of hinokinin series was pyrethroids 0.125%, synergist 1.00%, Triton X-100 34.00% and butyl acetate 64.875%, similarly the same of *d*-sesamin series was pyrethroids 0.25%, synergist 2.00%, Triton X–100 45.00% and xylene 52.75%. Each emulsion was tested six or ten kinds of the concentration and 100 mosquito larvae in ten petri-dishs were used for each concentration. The kill was recorded 24 hrs. after treatment.

Knockdown test against mosquito larvae The knockdown effectiveness of pyrethroids-synergists combined (1:8) emulsions against mosquito larvae were evaluated by means of petri-dish method which was previously described by the author.[8] One hundred individuals in ten petri-dish were used for each experiment. The cumulative numbers of knockdowned insects under the bottom of petri-dish during from one to sixty four min. was recorded after treatment.

RESULTS AND DISCUSSION

The lethal and knockdown toxicities tests of pyrethroids alone or with synergists against adults of houseflies and mosquito larvae followed the general design of probit analysis developed by Bliss.[9]

These dosage-mortality or time-knockdown regression equations of each tests showed in Tables 1, 3, 5 and 7.

The LD_{50}, LC_{50} or KT_{50} values of each toxicants calculated from regression equations, and the degree of synergism of each lignans and piperonyl butoxide for pyrethrins and allethrin calculated from LD_{50}, LC_{50} or KT_{50} of pyrethroids alone/ LD_{50}, LC_{50} or KT_{50} of pyrethroids with synergist were shown in Tables 2, 4, 6 and 8.

Taiwanin A was found to act as synergist for pyrethrins and allethrin likewise hinokinin or hibalactone which closely connected with this new lignan structurally, but its synergistic activities with both pyrethroids were less effective than that of hinokinin or hibalactone in the topical application and knockdown test against houseflies. In these tests, the observation of the fact that the combination of allethrin with taiwanin A was more effective than the similar combination of pyrethrins, was coincided with the results of the previous papers by the author[10] concerned with hibalactone or hinokinin.

From these results, it was thought that the combination of the central nucleus

Table 1. Dosage (μ g)-Mortality(%) Regression Equation of Pyrethrins and Allethrin Alone or Combined with Hinokinin, Hibalactone, Taiwanin A, *d*-Sesamin or Pawlownin at a Ratio of 1:8 Applied Topically to Female Houseflies.

Toxicant	Regression equation
Pyrethrins alone	Y=4.945+3.570(X−0.863)
+Hinokinin	Y=4.993+3.867(X−0.588)
+Hibalactone	Y=5.177+4.053(X−0.537)
+Taiwanin A	Y=5.244+5.749(X−0.867)
Allethrin alone	Y=4.999+3.160(X−1.057)
+Hinokinin	Y=5.313+3.535(X−0.603)
+Hibalactone	Y=5.427+4.064(X−0.589)
+Taiwanin A	Y=5.275+4.455(X−0.895)
Pyrethrins alone	Y=5.075+3.759(X−1.044)
+*d*-Sesamin	Y=5.024+2.770(X−0.408)
+Pawlownin	Y=5.226+3.541(X−0.924)
Allethrin alone	Y=4.418+4.446(X−1.044)
+*d*-Sesamin	Y=4.975+6.470(X−0.751)
+Pawlownin	Y=4.475+7.677(X−0.934)

Table 2. The LD_{50} Values of Pyrethrins and Allethrin Alone or Combined with Hinokinin, Hibalactone, Taiwanin A, *d*-Sesamin or Pawlownin at a Ratio of 1:8 Applied Topically to Female Houseflies and Degrees of Synergism for Lethal Effectiveness.

Toxicant	LD_{50} (μg/♀fly) within 24 hrs.	Degree of synergism	Ratio
Pyrethrins alone	0.752±0.010	—	—
+Hinokinin	0.389±0.000	1.93	1.00
+Hibalactone	0.311±0.006	2.41	1.25
+Taiwanin A	0.668±0.023	1.13	0.58
Allethrin alone	1.275±0.060	—	—
+Hinokinin	0.327±0.004	3.91	1.00
+Hibalactone	0.305±0.014	4.18	1.07
+Taiwanin A	0.681±0.014	1.87	0.48
Pyrethrins alone	1.056±0.048	—	—
+*d*-Sesamin	0.251±0.011	4.21	1.00
+Pawlownin	0.725±0.027	1.46	0.35
Allethrin alone	1.495±0.007	—	—
+*d*-Sesamin	0.569±0.031	2.56	1.00
+Pawlownin	1.001±0.000	1.49	0.58

Table 3. Time (min.)-Knockdown(%) Regression Equation of Pyrethrins and Allethrin Alone Dusts or Combined with Hinokinin, Hibalactone, Taiwanin A, *d*-Sesamin, Pawlownin or Piperonyl Butoxide at a Ratio of 1:8 by Settling Dust Appratus Method against Houseflies.

Toxicant conc.(%) in dust Pyrethroid		Synergist	Regression equation
Pyrethrins	0.058		Y=4.743+1.278(X−1.126)
	+Hinokinin	0.466	Y=4.773+1.193(X−0.716)
	+Hibalactone	0.466	Y=4.836+1.634(X−0.703)
	+Taiwanin A	0.466	Y=5.184+1.315(X−1.102)
	+Pip. But.	0.466	Y=5.921+2.736(X−0.519)
Allethrin	0.035		Y=5.053+1.167(X−1.212)
	+Hinokinin	0.280	Y=5.805+7.195(X−0.373)
	+Hibalactone	0.280	Y=4.910+5.322(X−0.472)
	+Taiwanin A	0.280	Y=5.253+2.698(X−0.739)
	+Pip. But.	0.280	Y=5.214+3.626(X−0.651)
Pyrethrins	0.025		Y=4.698+1.915(X−1.104)
	+*d*-Sesamin	0.200	Y=4.680+2.343(X−0.829)
	+Pawlownin	0.200	Y=4.770+2.260(X−0.988)
	+Pip. But.	0.200	Y=5.007+4.299(X−0.737)
Allethrin	0.021		Y=4.403+3.165(X−0.826)
	+*d*-Sesamin	0.166	Y=4.373+3.297(X−0.868)
	+Pawlownin	0.166	Y=5.000+3.058(X−0.914)
	+Pip. But.	0.166	Y=4.966+4.170(X−0.773)

Table 4. The KT_{50} Values of Pyrethrins and Allethrin Alone Dusts or Combined with Hinokinin, Hibalactone, Taiwanin A, *d*-Sesamin, Pawlownin or Piperonyl Butoxide at a Ratio of 1:8 by Settling Dust Apparatus Method against House-flies and Degree of Synergism for Knockdown Effectiveness.

Toxioant	KT_{50} (min.)	Degree of synergism	Ratio
Pyrethrins alone	21.257±0.189	—	—
+Hinokinin	6.848±0.012	3.10	1.00
+Hibalactone	6.363±0.083	3.34	1.08
+Taiwanin A	9.178±0.003	2.32	0.75
+Pip. But.	1.520±0.002	13.98	4.50
Allethrin alone	14.673±0.066	—	—
+Hinokinin	1.821±0.007	8.03	1.00
+Hibalactone	3.083±0.003	4.76	0.59
+Taiwanin A	4.924±0.013	2.98	0.38
+Pip. But.	3.907±0.055	3.76	0.47
Pyrethrins alone	18.274±0.457	—	—
+*d*-Sesamin	9.230±0.157	1.98	1.00
+Pawlownin	12.303±0.369	1.49	0.75
+Pip. But.	5.434±0.109	3.36	1.70
Allethrin alone	10.351±0.279	—	—
+*d*-Sesamin	8.860±0.018	1.17	1.00
+Pawlownin	8.197±0.082	1.26	1.08
+Pip. But.	6.041±0.272	1.71	1.47

Table 5. Concentration (ppm)-Mortality(%) Regression Equation of Pyrethrins and Allethrin Alone Emulsions or Combined with Hibalactone, Taiwanin A, *d*-Sesamin, Pawlownin or Piperonyl Butoxide at a Ratio of 1:8 by Petri-dish Method against Mosquito Larvae.

Toxicant	Regression equation
Pyrethrins alone	Y=4.810+4.527(X−1.169)
+Hibalactone	Y=4.958+6.860(X−1.042)
+Taiwanin A	Y=4.986+6.309(X−1.119)
Pyrethrins alone	Y=5.125+13.274(X−1.610)
+Taiwanin A	Y=4.944+11.762(X−1.403)
+Pip. But.	Y=4.861+16.381(X−1.368)
Allethrin alone	Y=5.053+6.857(X−1.875)
+Hibalactone	Y=5.156+8.038(X−1.540)
+Taiwanin A	Y=4.959+7.014(X−1.724)
Allethrin alone	Y=4.547+6.828(X−2.160)
+Taiwanin A	Y=4.995+16.189(X−1.961)
+Pip. But.	Y=5.080+17.964(X−2.069)
Taiwanin alone	Y=4.822+5.246(X−2.677)
Pip. But. alone	Y=4.935+4.694(X−2.732)
Pyrethrins alone	Y=5.094+4.996(X−0.690)
+*d*-Sesamin	Y=4.894+5.453(X−0.506)
+Pawlownin	Y=4.922+6.036(X−0.590)
Allethrin alone	Y=5.264+3.498(X−1.428)
+*d*-Sesamin	Y=4.617+4.908(X−1.111)
+Pawlownin	Y=4.991+6.423(X−1.325)
d-Sesamin alone	Y=4.919+7.295(X−1.771)
Pawlownin alone	Y=4.833+5.208(X−1.749)

Table 6. The LC_{50} Values of Pyrethrins and Allethrin Alone Emulsion or Combined with Hibalactone, Taiwanin A, *d*-Sesamin, Pawlownin or Piperonyl Butoxide at a Ratio of 1:8 by Petri-dish Method against Mosquito Larvae and Degrees of Synergism for Lethal Effectveness.

Toxicant	LC_{50} (ppm as stock emulsion) within 24 hrs.	Degree of synergism	Ratio
Pyrethrins alone	16.25±0.02	—	—
+Hibalactone	11.16±0.05	1.46	1.00
+Taiwanin A	13.22±0.08	1.23	0.84
Pyrethrins alone	39.87±1.86	—	—
+Taiwanin A	25.57±0.18	1.56	1.00
+Pip. But.	23.80±0.72	1.68	1.07
Allethrin alone	73.67±0.42	—	—
+Hibalactone	33.19±0.58	2.22	1.00
+Taiwanin A	53.75±0.58	1.37	0.62
Allethrin alone	168.39±0.27	—	—
+Taiwanin A	91.39±3.18	1.84	1.00
+Pip. But.	116.00±0.00	1.45	0.79
Taiwanin A alone	513.61±5.11	—	—
Pip. But. alone	556.85±6.45	—	—
Pyrethrins alone	4.69±0.06	—	—
+*d*-Sesamin	3.35±0.00	1.40	1.00
+Pawlownin	4.01±0.04	1.17	0.84
Allethrin alone	22.52±0.86	—	—
+*d*-Sesamin	15.47±0.13	1.80	1.00
+Pawlownin	21.22±0.50	1.32	0.73
d-Sesamin alone	60.65±0.43	—	—
Pawlownin alone	60.46±0.72	—	—

Table 7. Time (min.)-Knockdown(%) Regression Equation of Pyrethrins and Allethrin Alone Emulsion or Combined with Hinokinin, Hibalactone, Taiwanin A, *d*-Sesamin, Pawlownin or Piperonyl Butoxide at a Ratio of 1:8 by Petri-dish Method against Mosquito Larvae.

Toxicant conc. (ppm) in emulsion tested Pyrethroid		Synergist	Regression equation
Pyrethrins	0.024		Y=4.557+1.147(X−1.256)
	+Hinokinin	0.190	Y=4.411+1.341(X−1.127)
	+Hibalactone	0.190	Y=4.646+1.502(X−1.169)
	+Taiwanin A	0.190	Y=4.765+1.428(X−1.156)
	+Pip. But.	0.190	Y=4.616+1.685(X−0.816)
Allethrin	0.130		Y=4.634+0.941(X−1.325)
	+Hinokinin	1.040	Y=5.104+1.579(X−1.195)
	+Hibalactone	1.040	Y=5.151+1.608(X−1.266)
	+Taiwanin A	1.040	Y=4.777+1.033(X−1.315)
	+Pip. But.	1.040	Y=5.300+2.196(X−1.407)
Pyrethrins	0.019		Y=4.727+2.536(X−1.474)
	+*d*-Sesamin	0.150	Y=4.941+2.107(X−1.522)
	+Pawlownin	0.150	Y=4.591+2.130(X−1.398)
Allethrin	0.100		Y=4.820+0.755(X−1.520)
	+*d*-Sesamin	0.800	Y=5.300+2.144(X−1.411)
	+Pawlownin	0.800	Y=5.226+1.440(X−1.427)

Table 8. The KT_{50} Values of Pyrethrins and Allethrin Alone Emulsion or Combined with Hinokinin, Hibalactone, Taiwanin A, *d*-Sesamin, Pawlownin or Piperonyl Butoxide at a Ratio of 1:8 by Petri-dish Method against Mosquito Larvae and Degrees of Synergism for Knockdown Effectiveness.

Toxicant	KT_{50} (min.)	Degree of synergism	Ratio
Pyrethrins alone	43.87±0.05	—	—
+Hinokinin	36.82±0.07	1.19	1.00
+Hibalactone	25.37±0.09	1.73	1.45
+Taiwanin A	19.97±0.06	2.20	1.84
+Pip. But.	11.06±0.01	3.97	3.33
Allethrin alone	51.70±0.04	—	—
+Hinokinin	13.47±0.01	3.84	1.00
+Hibalactone	14.85±0.08	3.48	0.91
+Taiwanin A	33.99±0.01	1.52	0.40
+Pip. But.	18.84±0.02	2.77	0.72
Pyrethrins alone	39.83±0.45	—	—
+*d*-Sesamin	35.52±0.16	1.12	1.00
+Pawlownin	38.90±0.15	1.02	0.91
Allethrin	57.45±0.12	—	—
+*d*-Sesamin	8.70±0.09	3.07	1.00
+Pawlownin	18.64±0.02	3.08	1.00

such as γ-lactone ring to the carbon chain between two 3,4-methylenedioxyphenyl groups was a necessary condition in order to more synergize allethrin than pyrethrins.

Furthermore, in the knockdown test against houseflies, the synergistic activities of taiwanin A with either pyrethrins or allethrin were less effective than that of piperonyl butoxide. In the lethal test against mosquito larvae, the synergistic activities of taiwanin A with pyrethrins and allethrin were also less effective than that of hibalactone, whereas this activity of taiwanin A with pyrethrins was less effective than that of piperonyl butoxide in spite of the same with allethrin was more effective than that of piperonyl butoxide. In the knockdown test against mosquito larvae, the synergistic activity of taiwanin A with pyrethrins was most effective among three lignans, and these activities increased as double bond increases, but the activity of taiwanin A was far less effective than that of piperonyl butoxide. On the contrary, the synergistic activity of taiwanin A with allethrin was least effective among three lignans and these activities decreased as double bond increases, furthermore the activity of taiwanin A was less effective than that of piperonyl butoxide.

From these experimental results, it was concluded that the introduction of two double bonds into the carbon chain between two of 3,4-methylenedioxyphenyl groups causes the decrease of the synergistic activity with pyrethrins and allethrin in spite of the same of one double bond causes increase in the synergistic activity except in the case of the knockdown effectiveness of pyrethrins against mosquito larvae.

In the lethal tests against houseflies and mosquito larvae, the synergistic activities of pawlownin with either pyrethrins or allethrin were less effective than that of *d*–sesamin. In the knockdown test against houseflies, the synergistic activity of pawlownin with pyrethrins was appreciably less effective than that of *d*-sesamin and piperonyl butoxide, but the synergistic activity of pawlownin with allethrin was more effective than that of *d*-sesamin although its activity was less effective than that of piperonyl butoxide. In the knockdown test against mosquito larvae, pawlownin was not synergistic with pyrethrins although allethrin was strongly synergized by pawlownin, furthermore its degree of synergism was competed with that of *d*-sesamin.

In general, the substitution of hydroxyl group into a central nucleus of 3,4–methylenedioxyphenyl bis-furanoid lignans such as *d*-sesamin causes of the decrease of the synergistic activities with pyrethrins and allethrin. These observation was coincided with the result reported by Beroza *et al.*[11]

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